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Significance of Groundnut Inoculation and NP Fertilizer Application on Yield, Nitrogen Uptake, Fixation and Soil N Balance Sheet under Rainfed Conditions

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Abstract
A study was carried out at two different locations in northern Punjab-Pakistan to elucidate the effect of inoculum and fertilizer application on growth and nitrogen fixation of two varieties (BARI 2000 & Golden) of groundnut (*Arachis hypogea*) under rainfed conditions. The treatments comprised of different combinations of two levels of nitrogen (0 and 20 kg ha^{-1}) and two levels of phosphorus (0 and 80 kg P_2O_5 ha^{-1}) with and with out inoculation. Application of fertilizer and inoculum resulted in significantly higher dry matter yield as compared to control at both the locations, however effect was not significant on pod yield. The percent nitrogen derived from atmosphere was in range of 32 to 49 and was not significantly affected by either fertilizer or inoculum application. Amount of nitrogen fixed increased by 40% at Chakwal and by 11% at Talagang due to fertilizer combination of 20 kg nitrogen and 80 kg phosphorus ha^{-1}.
Keywords: Arachis hypogeal, Inoculation, Pod yield, N fixation, N balance

Introduction

Peanut (*Arachis hypogea*) is an important cash crop of Pakistan and is grown on an area of about 92.8 thousand hectares with production of 85.5 thousand tonnes (Government of Pakistan, 2010). The average yield is 921 kg ha^{-1} which is much lower than developed countries of the world such as China (3357 kg ha^{-1}), USA (3824 kg ha^{-1}) and Israel (5598 kg ha^{-1}) (FAO, 2009).

Nitrogen (N) is deficient in all of our soils where as phosphorus (P) is deficient in 90% soils (Ahmad and Rashid, 2003). However, use of inorganic fertilizers for groundnut is very limited. Nitrogen is constituent of all proteins and enzymes, chlorophyll, several intermediates in metabolic synthesis and deoxyribonucleic acid. Phosphorus application significantly affects many aspects of plant physiology including photosynthesis, flowering, fruiting and maturation which ultimately results in better chickpea yield (Brady and Weil, 2005). It is also essential component of nucleic acids, phosphatides, phospholipids, chromosomes, coenzymes, nicotinamide adenine dinucleotide and adenosine tri phosphate (Ahmad and Rashid, 2003).

The effect of the level of available N in the soil on symbiotic N fixation varies with legume species and environmental factors. Generally, high levels of available N in the soil depress N fixation (Biederbeck et al., 1996). However, due to the lag period between Rhizobium colonization and the onset of nodule functioning, the young legume plants require adequate N from external sources in order to achieve proper vegetative growth and establishment of the N fixing symbiosis (Kucey, 1989). A fine line separates excess available N that may depress N fixation and sufficient available N to enhance N fixation.

The nitrogen fixing plants of legumes requires P for adequate growth and nodulation. The process of N fixation is very sensitive to the availability of nutrient especially N and P (Tang et al., 2001). The influence of phosphorous on symbiotic nitrogen fixation in leguminous plants has received considerable attention but its role in the process remains unclear (Tsvetkova and Georgiev, 2003). Some researchers are of opinion that it is directly involved in the process while other others are of opinion that it increases overall plant growth (Shu Jie et al., 2007).

Poor plant population arising from less soil moisture at the time of germination, inadequate fertilizer use, use of inefficient rhizobial strain, poor plant protection measures and poor post harvest technology are among major constraints for low groundnut yield. The rhizobial inoculation has been paid little attention among these constraints although significant increase in the growth and yield of important crops due to inoculation with rhizobacteria is documented by various researchers (Fatima et al., 2007).

Therefore keeping in view all these facts, a trial was conducted to study the effect of inoculation and different combinations of N and P fertilizers on N fixation and yield of groundnut under rainfed conditions of Pakistan.

Materials and Methods

The field experiments were carried out at two different locations (Barani Agricultural Research Institute, Chakwal and at farmer's field Talagang) under rainfed conditions in northern Punjab, Pakistan during summer 2006. The physical and chemical characteristics of the experimental sites are given in the Table 1. The treatments comprised of two varieties of groundnut (BARI 2000 and Golden), four combinations of N and P (F_1: 0-0, F_2: 20-0, F_3: 0-80, F_4:20-80 kg ha^{-1}) with and with out inoculum application. Nitrogen was applied in the form of urea and phosphorus in the form of triple super phosphate as basal dose. The rhizobial inoculant NC-92 was obtained from Land Resources Research Institute, National Agricultural Research Centre, Islamabad and was applied to the seed prior to sowing. The application was carried out by first making slurry with water, sugar and the inoculum and then shaking the seed in that slurry for a few minutes, after that sowing was carried out. The experiment was laid out according to randomized complete block design with three factors factorial arrangement. The net plot size was 6 × 2.7 m^2 at Talagang and it was 4 × 2 m^2 at Chakwal. The plant × plant distance was 10 cm and the row × row distance was 45 cm. Crop was grown under rainfed conditions and no supplemental irrigation was applied. There was no incidence of pest or disease attack so no chemical was sprayed. There were approximately 10 to 12 plants in an area of one square meter. There were 30 to 40 nodules per plant as indicated by plant sampling prior to flowering. Rainfall data for both the locations were recorded (Table 2). Harvesting was done during last week of October. Crop from an area of one meter square in the middle of each plot was harvested separately. The plant samples were sun dried and data were recorded for straw and grain yield. Representative samples of 100 gram for both straw and grain separately were collected from bulk sample, oven dried, ground and were chemically analyzed for N (Ryan et al., 2001). Sample of plant dry matter tissue of legume and non legume reference plant were also taken for δ^{15}N determination by mass spectrometer (Unkovich et al., 2008).

Percent nitrogen derived from air (% N_{dfa}) =100 × (δ^{15}N (soil N) **-** δ^{15}N legume N)/ (δ ^{15}N (soil N)-B) where δ^{15}N (soil N) is commonly obtained from a non N fixing reference plant grown in the same soil as the legume; B is the δ^{15}N of the same N fixing plant when fully dependent on N fixation and its value is -2.0 (Kyei-Boahen et al., 2002).

Legume N uptake (kg ha^{-1}) = legume dry matter yield (kg ha^{-1}) × N in plant tissue (%)

Amount of N fixed (kg ha^{-1}) =legume N uptake (kg ha^{-1}) × % N_{dfa}

Data on all observations were subjected to analysis of variance (ANOVA) by using software MSTATC. Treatment means were compared by Least Significant Difference (LSD) test. Correlation analysis was also done to study the relationship between different parameters.

Results

Pod and dry matter yield

Different fertilizer application rates were statistically similar to each other in respect of pod yield; however, inoculation resulted in significant increase in pod yield at both the locations (Table 3). Both the varieties of groundnut were similar in respect of pod yield. Nitrogen and phosphorus application resulted in significant increase in dry matter yield as compared to control at both the locations. Difference between two varieties in respect of dry matter yield and harvest index was not significant; however inoculum application resulted in significant increase in dry matter yield at both the locations. The variety by fertilizer interaction was significant in respect of dry matter yield. Significantly higher dry matter yield of Golden variety was recorded due N application as compared to BARI 2000 (Fig. 1). Application of P resulted in increase in harvest index at Chakwal where as no consistent trend was observed at Talagang (Table 3). Pod yield and dry matter yield was higher at Talagang as compared to Chakwal while harvest index was lower at Talagang as compared to Chakwal. The fertilizer by variety interaction was significant in respect of harvest index where significantly higher harvest index was recorded for BARI 2000 in F1 (20 kg N ha^{-1}) (Fig. 2).

Nitrogen fixation and uptake

Two groundnut varieties were statistically similar in respect of % N_{dfa}, N fixation and uptake (Table 4). Effect of inoculation was not significant on % $N_{dfa;}$ however, there was significant increase in N fixation and uptake with inoculation at both the locations. Effect of fertilizer application was non significant on % N_{dfa} and N uptake. The fertilizer × variety interaction in respect of N uptake was significant at Chakwal. Nitrogen uptake by groundnut variety Golden was significantly higher as compared to BARI 2000 in F1 (20 kg N ha^{-1}) and F3 (20 kg N + 80 kg P_2O_5 ha^{-1}) (Fig. 3). There was significant increase in amount of N fixed due to fertilizer application at Chakwal (Table 4). Maximum amount of N was fixed in F3 (20 kg N + 80 kg P_2O_5 ha^{-1}) which was at par with F2 (80 kg P_2O_5 ha^{-1}) and minimum in control. The fertilizer × variety interaction was significant at Chakwal. Higher amount of N was fixed by Golden as compared to BARI 2000 in F1 (20 kg N ha^{-1}) and F3 (20 kg N + 80 kg P_2O_5 ha^{-1}) (Fig. 4).

Soil nitrogen balance

Soil nitrogen balance at the end of experiment was negative for all the treatments (Table 5). Soil nitrogen depletion was more at Talagang site as compared to Chakwal. Groundnut variety BARI 2000 resulted in more negative soil N balance as compared to golden variety at Talagang.

Discussion

Increase in dry matter yield due to N and P application is in line with the findings of Latif et al. (2008) and Islam et al. (2009). Main reason for increased dry matter yield due to N and P application is that application of fertilizer results in increased root growth resulting in increased area for water and nutrient absorption (Mafongoya et al., 2006). This may enhance the synthesis of assimilates which can result in increase in dry matter yield (Gobarah et al., 2006). Effect of fertilizer application on dry matter yield of groundnut was more pronounced as compared to pod yield. Reason might be that at reproductive stage during September and October especially at Talagang, there was drought which resulted in poor pod formation and low response to fertilizer application. It has been observed that low soil moisture availability is the biggest constraint to crop production in rainfed agriculture and generally fertilizer use efficiency or crop response to nutrient application is much lower under rainfed conditions as compared to irrigated agriculture (Sahi et al., 1997). In some of studies conducted under rainfed conditions, response to nutrient application is linked with moisture availability. Ramolemana (1999) declared that P fertilizer would always be beneficial under rainfed conditions if the soil water content during the two weeks after sowing is near field capacity. Groundnut crop is more sensitive to water stress during reproductive growth stage as compared to vegetative growth (Vurayai et al., 2011). Effect of fertilizer application had non significant effect on pod yield which is line with the finding of Hayat et al., (2010) who recorded non significant effect of application of 80 kg P_2O_5 ha^{-1} on grain yield of mung bean (*Vigna radiate*) and mash bean (*Vigna mungo*) under rainfed conditions. Dry matter yield was higher at Talagang due to light textured of soil which is more favorable for the growth of groundnut crop.

The effect of P application on % N_{dfa} was not significant while significant on N fixation. The present observations with chickpea regarding % N_{dfa} appear to support the finding that P deficiency affects the process of nitrogen fixation through its secondary effects on plant growth rather than direct involvement in nitrogenase functioning. Similar results regarding effect of P application on % N_{dfa} have been reported earlier in chickpea (1slam and Ali, 2009) and in white lupin (Schulze et al., 2006). This is also confirmed by strong positive correlation amount of nitrogen fixed with dry matter yield (r = 0.99**) and N uptake (r = 0.97**) and week correlation with % N_{dfa} (r= 0.45). However contrary to these findings, Hayat et al. (2008) reported an increase up to 32% in % N_{dfa} due to application of P (80 kg P_20_5 ha^{-1}) using mung bean and mash bean as test crop. There is an increase in nodule number and nitrogenase activity with P application which results in increase in % N_{dfa} (Fatima et al*.,* 2007). Similar to our findings, Tang et al. (2001) did not observe any role of P in nodule functioning and nitrogenase activity, but amount of N fixed increased with P application in bean (*Phaseolus vulgaris*). It seems that of role of P in nitrogen fixation varies with crop, growing conditions and time of measurement.

Negative N balance was due to the fact that nitrogen input (fertilizer and nitrogen fixation) was not enough to meet crop demand. Second point is that N balance after legume harvest is positive when crop residues are returned to soil and only seed or grain is removed (Amanuel et al., 2000). Habtemichial et al. (2007) reported positive soil N balance in range of 12 to 52 kg ha^{-1} after harvest of faba bean crop in Northern Ethiopia but major difference was that crop residues were returned to soil. There was negative correlation of all the parameters with soil N balance which shows that accelerated plant growth resulted in depletion of soil N reserves. Soil nitrogen balance was more negative at Talagang as compared to Chakwal due to higher dry matter yield.

Conclusion

Application of inoculum and fertilizers resulted in significant increase in the dry matter yield and nitrogen fixation by groundnut. However, phosphorus is not directly involved in the process of nitrogen fixation and increase in total nitrogen uptake as a result of accelerated plant growth leads to an increase in amount of nitrogen fixed. The process of nitrogen fixation is not enough to fulfill the crop demand and there will be depletion of soil nitrogen if crop residues are not returned to soil. Further experimentation is needed to work out the suitable combination of nitrogen and phosphorus to get maximum profit from groundnut crop under rainfed conditions.

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Table I: Physical and chemical properties of soils before start of experiments at two sites (0-15 cm)

Characteristics	**Chakwal Site**	**Talagang Site**
% Clay	21	15
% Silt	24	26
% Sand	55	59
Soil Texture	Sandy clay loam	Sandy loam
Soil pH	7.7	7.8
EC_e (dSm^{-1})	0.25	0.20
Available P (mg Kg^{-1})	2.4	2.0
Extractable K (mg Kg^{-1})	110	130
Nitrate nitrogen (mg Kg^{-1})	5.3	2.3
Bulk Density (g cm^{-3})	1.42	1.65

Table 2: Rainfall data (mm) for two locations during year 2006

Months	**Chakwal**	**Talagang**
April	8	0
May	16	18
June	135	23
July	109	109
August	72	96
Sept	52	10
October	0	0
Total	**392**	**256**

Table 3: Pod and dry matter yield and harvest index as function of variety, fertilizer and inoculum

Effect	Pod yield (Mg ha^{-1})		Dry matter yield (Mg ha^{-1})		Harvest Index (%)	
	Chakwal	Talagang	Chakwal	Talagang	Chakwal	Talagang
Variety (V)						
V1BARI 2000	1.9	4.2	4.0 b	16.1	47	27
V2 Golden	2.1	3.7	4.3 a	14.5	51	26
Significance level	NS	NS	NS	NS	NS	NS
Fertilizer (F)						
F0 (control)	2.1	3.7	3.7 b	13.5 c	42 b	28 a
F1 (20 kg N ha^{-1})	1.8	4.0	4.2 a	14.5 b	43 b	28 a
F2 (80 kg P_2O_5 ha^{-1})	2.1	3.8	4.3 a	17.3 a	48 a	23 b
F3 (20 kg N + 80 kg P_2O_5 ha^{-1})	2.1	4.2	4.4 a	15.8 ab	49 a	27 a
Significance level	NS	NS	**	**	*	*
Inoculum (I)						
uninoculated	1.6 b	3.5 b	3.4 b	12.5 b	49	28 b
inoculated	2.4 a	4.4 a	4.8 a	18.1 a	50	25 a
Significance level	**	**	**	**	NS	*
Interactions						
I x V	NS	NS	NS	NS	NS	NS
I x F	NS	NS	NS	NS	NS	NS
F xV	NS	NS	**	NS	**	NS
I x V x F	NS	NS	NS	NS	NS	NS

Means with different letters differ significantly according to Least Significant Difference (LSD) test (*P<0.05*). NS stands for non significant difference, * and ** denote significance at P < 0.05 and P < 0.01 levels, respectively.

Table 4: Percent nitrogen derived from atmosphere and nitrogen uptake and fixation as function of variety, fertilizer and inoculum

Effect	Percent nitrogen derived from atmosphere		Nitrogen uptake (kg ha^{-1})		Amount of nitrogen fixed (kg ha^{-1})	
	Chakwal	Talagang	Chakwal	Talagang	Chakwal	Talagang
Variety (V)						
BARI 2000	34.4	48.9	97	349	32.5	162
Golden	35.4	47.5	105	309	36.1	150
Significance level	NS	NS	NS	NS	NS	NS
Fertilizer (F)						
F0 (control)	33.0	48.6	93	311	29.8 c	149 b
F1 (20 kg N ha^{-1})	32.7	47.4	101	311	32.4 bc	153 b
F2 (80 kg P_2O_5 ha^{-1})	33.4	48.6	106	349	33.1 b	159 ab
F3 (20 kg N + 80 kg P_2O_5 ha^{-1})	40.5	48.1	105	345	41.8 a	165 a
Significance level	NS	NS	NS	NS	*	*
Inoculum (I)						
uninoculated	37.1	48.3	83 b	284 b	30.3 b	132 b
inoculated	32.7	48.1	119 a	374 a	38.3 a	180 a
Significance level	NS	NS	**	**	**	**
Interactions						
I x V	*	NS	NS	NS	NS	NS
I x F	NS	NS	NS	NS	NS	NS
F xV	NS	NS	**	NS	*	NS
I x V x F	NS	NS	NS	NS	NS	NS

Means with different letters differ significantly according to Least Significant Difference (LSD) test ($P<0.05$). NS stands for non significant difference, * and ** denote significance at $P < 0.05$ and $P < 0.01$ levels, respectively.

Table 5: Nitrogen uptake from soil and soil nitrogen balance as function of variety, fertilizer and inoculum

Effect	Amount of nitrogen taken from soil (kg ha^{-1})		Soil nitrogen balance (kg ha^{-1})	
	Chakwal	Talagang	Chakwal	Talagang
Variety (V)				
BARI 2000	64.5	187	-54.5	-177
Golden	68.9	159	-58.9	-149
Significance level	NS	NS	-	-
Fertilizer (F)				
F0 (control)	63.2	162 c	-63.2	-162
F1 (20 kg N ha^{-1})	68.6	158 c	-48.6	-138
F2 (80 kg P_2O_5 ha^{-1})	72.9	190 a	-72.9	-190
F3 (20 kg N + 80 kg P_2O_5 ha^{-1})	63.2	180 b	-43.2	-160
Significance level	NS	*	-	-
Inoculum (I)				
uninoculated	52.7 b	152 b	-42.7	-142
inoculated	80.7 a	194 a	-70.7	-184
Significance level	*	*	-	-

Means with different letters differ significantly according to Least Significant Difference (LSD) test ($P<0.05$). NS stands for non significant difference, * and ** denote significance at $P < 0.05$ and $P < 0.01$ levels, respectively.

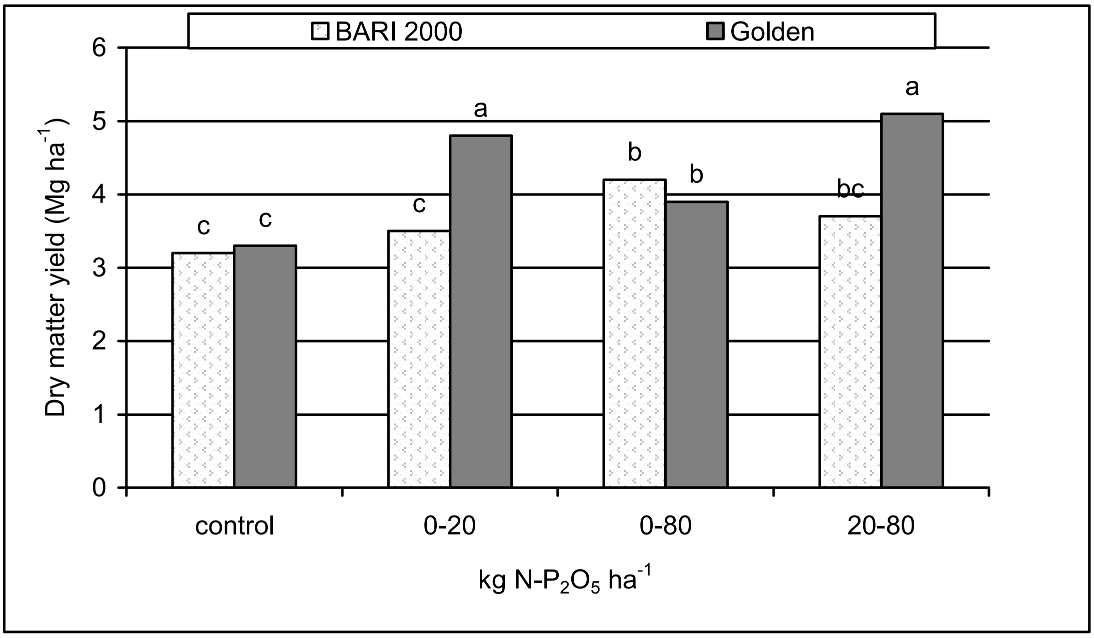

Fig. 1: Effect of inorganic fertilizers and varieties interaction on dry mater yield of groundnut at Chakwal

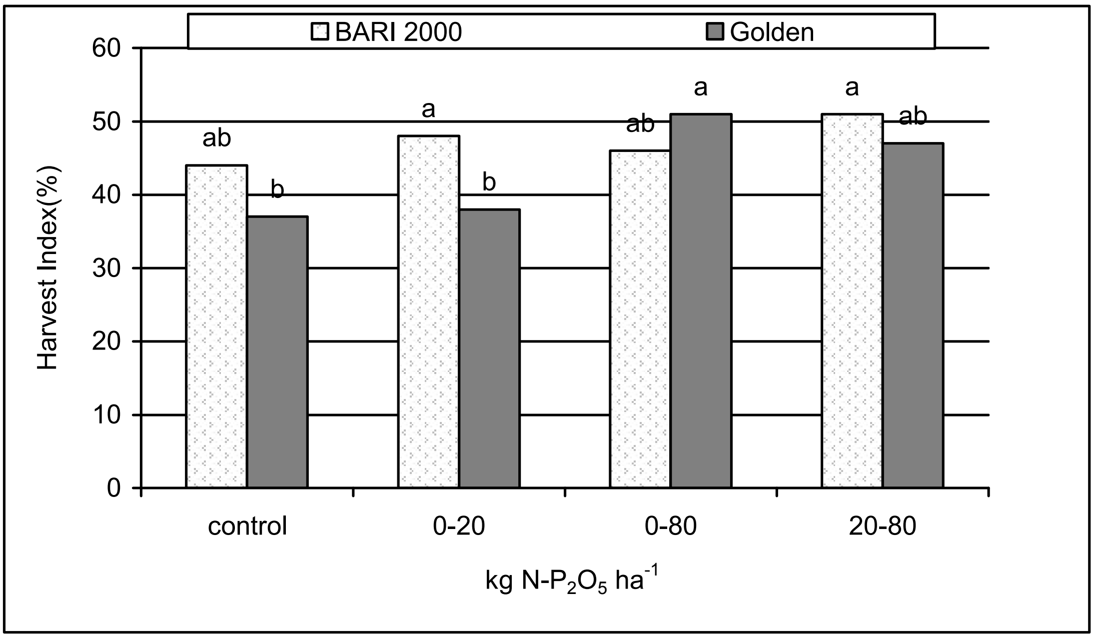

Fig. 2: Effect of inorganic fertilizers and variety interaction on harvest index of groundnut at Chakwal

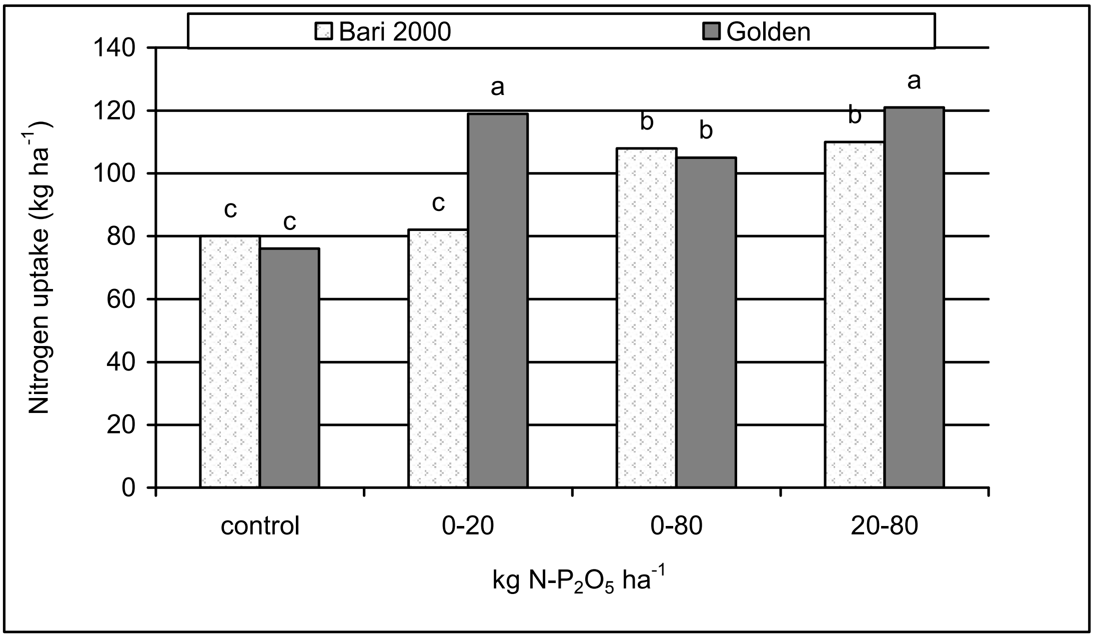

Fig. 3: Effect of inorganic fertilizers and variety on nitrogen uptake by groundnut at Chakwal

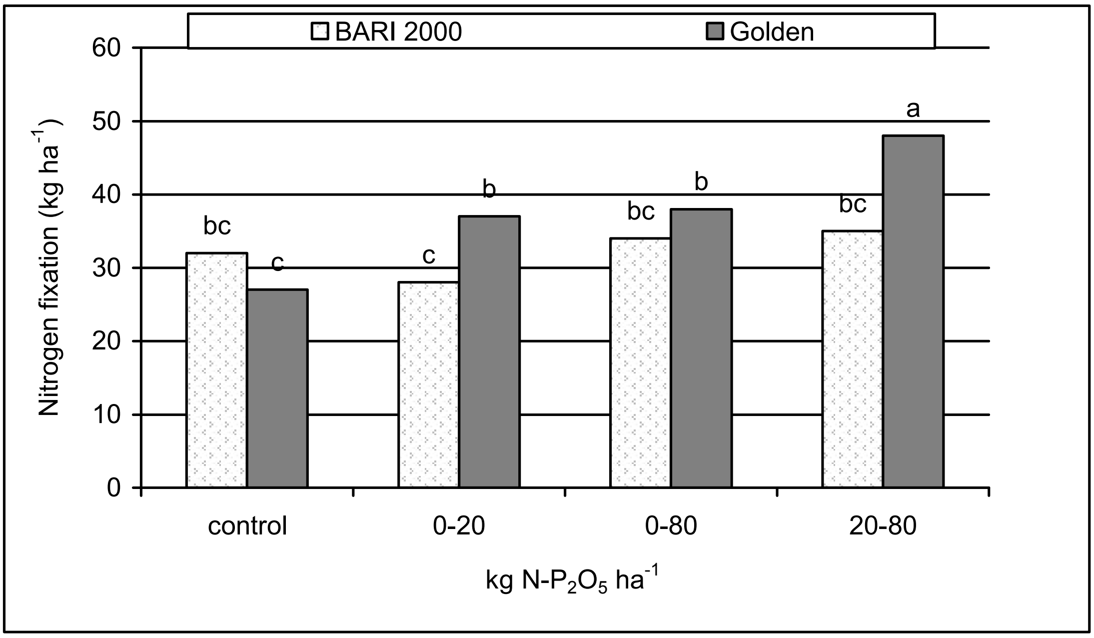

Fig. 4: Inorganic fertilizers and variety interaction on nitrogen fixation by groundnut at Chakwal